Bones in balance

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Chapter 5

Gender-affirming hormone treatment decreases bone turnover in trans women and older trans men

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Abstract

Sex steroids play a key role in bone turnover and preserving BMD. Gender-affirming hormonal treatment (HT) in transgender people affects bone metabolism. Most studies looked into the effect of HT on changes in BMD, however this does not provide insights of changes in bone metabolism due to HT. This study investigated changes in bone turnover markers (BTMs), sclerostin and correlations with change in BMD in trans women and trans men during the first year of HT. Trans women received estradiol and anti-androgens, while trans men received testosterone. Sclerostin, P1NP, alkaline phosphatase (ALP), CTx, and BMD of total hip (TH), femoral neck (FN), and lumbar spine (LS) were evaluated at baseline and after 1 year of HT. 121 Trans women (median age 30 years, IQR 24-41) and 132 trans men (median age 24 years, IQR 21-33) were included. In trans women, ALP decreased with 19% (95%CI -21;-16), CTx with 11% (95%CI -18;-4) and sclerostin with 8% (95%CI -13;-4) after 1 year of HT. In contrast, in trans men P1NP, ALP, and sclerostin increased with 33% (95%CI 24;42), 16% (95%CI 12;20), and 15% (95%CI 10;20), respectively after 1 year of HT. No age differences were seen in trans women, whereas in trans men aged ≥50 years a decrease in all BTMs was found in contrast to the other age groups. These trans men had low estrogen concentration at start of HT, due to their postmenopausal state before start of HT, and estradiol concentrations increased during testosterone treatment. Changes in BTMs and BMD were weakly correlated (correlation coefficient all <0.30). To conclude, 1 year of HT resulted in decreased bone turnover in trans women and older trans men, while it increased in younger trans men. The decrease in bone resorption in the older trans men displays the importance of estrogen as key regulator of bone turnover.

Introduction

Sex steroids are considered as pivotal regulators of bone metabolism. Estrogen inhibits the osteoclast function and thereby lowers bone resorption, resulting in a positive effect on BMD in both women and men [1–5]. Furthermore, it is well known that BMD decreases in postmenopausal women due to decreasing estrogen concentrations and consequently increased bone resorption by osteoclasts [6,7]. In men, estrogen is aromatized from testosterone and is also considered as the key sex steroid affecting bone homeostasis [8–10]. Previous research showed that bone metabolism and therefore BMD are affected by gender-affirming hormonal treatment (HT) in people diagnosed with gender dysphoria (GD) [5,11–18]. HT is used to accomplish desired body changes in transgender people. HT in trans men (female-to-male transgender people) consists of testosterone treatment, while trans women (male-to-female transgender people) receive a combination of anti-androgens and estrogens.

An increase in BMD after 1 to 10 years of treatment with HT in transgender people was described before [5,19]. BMD is evaluated by DXA scan, however these scans estimate the amount of mineralized bone only and therefore represent late changes in bone metabolism. In contrast, bone turnover markers (BTMs) represent the actual activity of the osteoblasts and osteoclasts. Consequently, measurements of BTMs display the balance between bone formation and bone resorption directly. Up until now, scarce data is available regarding specifically the effect of HT on bone turnover in transgender people [20–24], while no data on sclerostin is available yet. Clinically, increased bone turnover and lower BMD are risk factors for deterioration of bone quality resulting in possible osteopenia, osteoporosis, and even increased risk of fractures and associated co-morbidities and financial costs. As the transgender population receiving HT increases worldwide [25,26], more transgender people are possibly at risk for lower bone quality and associated problems regarding bone health. Therefore, the aim of this study is to investigate the change in bone turnover markers and to evaluate the correlations with changes in BMD in adult transgender people during their first year of HT.

This study will also look into possible age-related effects on bone turnover markers and BMD during HT by studying transgender people in various age groups. We hypothesize to find a decrease in bone turnover predominantly due to estrogen, as this sex steroid is known to inhibit osteoclast function and therefore exerts an anabolic effect on bone. This study will focus on bone formation markers P1NP and total alkaline phosphatase (ALP) and the bone resorption marker CTx. Furthermore, the glycoprotein sclerostin is studied, which is known to mediate an anti-anabolic effect on bone by promoting apoptosis of osteoblasts, stimulate RANKL production by osteocytes resulting in increased osteoclastogenesis, and inhibition of the activation of the Wnt/β-catenin pathway, all resulting in negative effects on BMD [27–32]. Sclerostin is mainly produced by osteocytes and can be used as a marker of bone metabolism as well.

Methods

Subjects and study protocol

This study is part of the European Network for Investigation of Gender Incongruence (ENIGI) study, which is a prospective multicenter observational study in Ghent (Belgium), Oslo (Norway), Florence (Italy), and Amsterdam (the Netherlands) [33,34]. The current study protocol was approved by the Ethical Committee of the Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands, and data was retrieved only after informed consent. Adults diagnosed with gender dysphoria based on the diagnostic criteria of the DSM-IV or DSM 5 [35,36] were recruited at the Center of Expertise on Gender Dysphoria of the Amsterdam University Medical Center, between June 2012 and April 2016. All transgender people included in this study were treated according to the Standards of Care Guidelines of the World Professional Association for Transgender Health (WPATH) [37]. Summarized, trans women were treated with anti-androgen treatment consisting of cyproterone acetate (50 to 100 mg daily, oral) accompanied by estrogen treatment consisting of either estradiol valerate (2 to 4 mg daily, oral) or estradiol patches (50 - 100 mcg/24 h twice a week, transdermal application). Trans men were treated with either testosterone gel (50 mg daily, dermal application), testosterone esters (250 mg every 2 to 3 weeks, i.m.), or testosterone undecanoate (1000 mg every 12 weeks, i.m.). Some trans men used lynestrenol for a short period of time if menses persisted while using testosterone.

People were not eligible to participate in the study if they had (I) insufficient knowledge of the native language, (II) were psychological vulnerable, (III) used HT earlier in life, or (IV) used other drug therapies which were not part of the standardized treatment protocol (e.g. spironolactone or gonadotropin-releasing hormone agonists). For the current analyses, people were excluded if they (I) did not completed 1 year of HT, (II) had no DXA scan at baseline and/or after 1 year of HT, (III) or had no blood drawn at baseline and after 1 year of HT. In addition, only people from the Amsterdam University Medical Center, Vrije Universiteit Amsterdam center were included, in order to exclude possible changes in BTM concentrations due to use of different BTM assays in the other medical centers.

Measurements

General

Participants visited the outpatient clinic every 3 months to evaluate their health and treatment effects. Body weight (kilograms) and height (centimeters) were measured without wearing shoes at baseline and follow-up. Blood samples were collected between 09:00 AM and 12:00 PM at baseline, after 3 months, and after 1 year of HT. Participants were instructed to draw blood in a fasting state.

Bone turnover markers

P1NP

The bone formation marker P1NP resembles osteoblast activity [38] and was measured using an immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany), with an inter-assay coefficient of variation (CV) of <8% and lower limit of quantification (LOQ) of 5 µg/L.

Alkaline phosphatase (ALP)

The bone formation marker ALP, also representing osteoblast activity [38] was measured using an immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany), with an inter-assay CV of 2.5% and LOQ of 5 U/L.

CTx

The bone resorption marker CTx displays osteoclast activity [38]. CTx was measured using an immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany), with an inter-assay CV of <6.5% and LOQ of 10 ng/L.

Sclerostin

The osteocyte-derived glycoprotein sclerostin [38] was measured using an immunoassay (LiasonXL, Diasorin, Saluggia, Italy), with an inter-assay CV of 7.5% and LOQ of 2.2 pmol/L.

Other measurements

25OHD

An LC-MS/MS method was used to measure 25OHD with an CV of 8% and LOQ of 4.0 nmol/L until 2015 [39]. Since then, another LC-MS/MS method was used [40]. Both methods resulted in comparable concentrations.

Testosterone

A radio-immunofrequent assay (RIA) (Coat-A-Count, Siemens, USA; inter-assay CV of 7-20 %, LOQ of 1 nmol/L) was used to measure testosterone until January 2013. Since then, a competitive immunoassay was used (Architect, Abbott, USA; inter-assay CV of 6-10 %, LOQ of 0.1 nmol/L). The RIA based concentrations were converted to concentrations of the competitive immunoassay using the formulas Architect=1.1*RIA+0.20 (for testosterone concentrations <8 nmol/L) and Architect=1.34*RIA–1.65 (for testosterone concentrations >8 nmol/L) in order to evaluate and report comparable testosterone concentrations.

Estradiol

A competitive immunoassay (Delfia, PerkinElmer, Finland; inter-assay CV of 10–13%, LOQ of 20 pmol/L) was used to measure estradiol until July 2014. Subsequently, an LC-MS/MS (Amsterdam University Medical Center, VUmc, Amsterdam, the Netherlands; inter-assay CV of <7%, LOQ of 20 pmol/L) was used. The Delfia concentrations were converted to the LC-MS/MS concentrations by using the formula LC-MS/MS=1.60*Delfia–29.

Creatinine, aspartate transaminase (AST), alanine transaminase (ALT), and gamma-glutamyltransferase (γGT) concentrations were all measured using an immunoassay (Cobas, Roche Diagnostics).

DXA

DXA (Hologic Discovery A, Hologic Inc., Bedford, MA, USA) was used to measure BMD in g/cm2 of the total hip (TH) and femoral neck (FN) of the non-dominant hip and the lumbar spine (LS), measuring the first 4 lumbar vertebrae (L1–L4). The software was updated from version 13.3 to 13.5.3 in July 2015, which did not affect the results of the measurements. Baseline DXA was performed 3 months before to 1 month after start of HT. The follow-up DXA was performed between 10 and 14 months after start of HT.

Statistics

For statistical analyses Stata/SE 15 (StataCorp, LP) was used. Median with corresponding interquartile range (IQR), percentages, or means with SD were used to describe baseline characteristics. The percentage change was calculated of all BTMs and BMD to evaluate differences between baseline and 1 year HT. As these changes were normally distributed, linear regression analyses were performed to evaluate mean changes in percent with corresponding 95% CI. Next, these percentage difference variables were adjusted for changes in BMI, alcohol and tobacco use, 25OHD, creatinine, AST, ALT, and -GT concentrations. Participants were stratified for both age and sex steroid concentrations, with the following age groups: 18 to 30 years, 30 to 50 years, and 50 years and older. By using these separate age groups, age related differences in BMD due to decreasing bone mass with increasing age after reaching peak bone mass is accounted for, as it is expected that bone mass decreases throughout time, as described before [5]. Linear regression was performed to evaluate possible differences between the separate age groups. Furthermore, participants were stratified into quartiles based on their mean estradiol and testosterone concentrations during HT, which were calculated by an average of the concentrations after 3 and 12 months of HT. This stratification was applied to detect possible differences between effect of either low or high sex steroid concentrations. Furthermore, a power analysis was performed. The analysis was applied to the study population of 121 trans women and 132 trans men in order to detect mean differences of both BMD and separate bone turnover markers with a power of 80% and alpha of 0.05. This resulted in detection of a mean difference of LS BMD of 0.021 g/cm2 in trans women and 0.022 g/cm 2 in trans men. Regarding bone turnover markers, in trans women a 10% change in CTx, 9% change in P1NP, 4% change in ALP, and 6% change in sclerostin could be detected. In trans men, a change of 10%, 13%, 6%, and 7% could be detected, respectively. Lastly, Pearson correlations were calculated between change in BTMs and BMD and are displayed with corresponding 95% CI.

Results

General

A total of 253 people were included in this study (Figure 1), which consisted of 121 trans women with median age of 30 (IQR 24 to 41) years and 132 trans men with median age of 24 (IQR 21 to 33) years. The baseline and follow-up characteristics are displayed in Table 1. In trans women, a median increase in estradiol of 129 pmol/L (IQR 56 to 232) implying a percentage change of estradiol of 128% (IQR 52 to 214) and a median decrease in testosterone of -18 nmol/L (IQR -22 to -14) with

a percentage decrease of -96% (IQR -97 to -94) was seen during the first year of HT. In trans men, a median increase in estradiol of 46 pmol/L (IQR -304 to 135) with a percentage change of 26% (IQR -63 to 198), which was accompanied by a median increase in testosterone of 27 nmol/L (IQR 20 to 38) and percentage increase of 2248% (IQR 1311 to 3338) was seen during the first year of HT.

In both groups, the BMI increased and tobacco use decreased during 1 year of HT (Table 1).

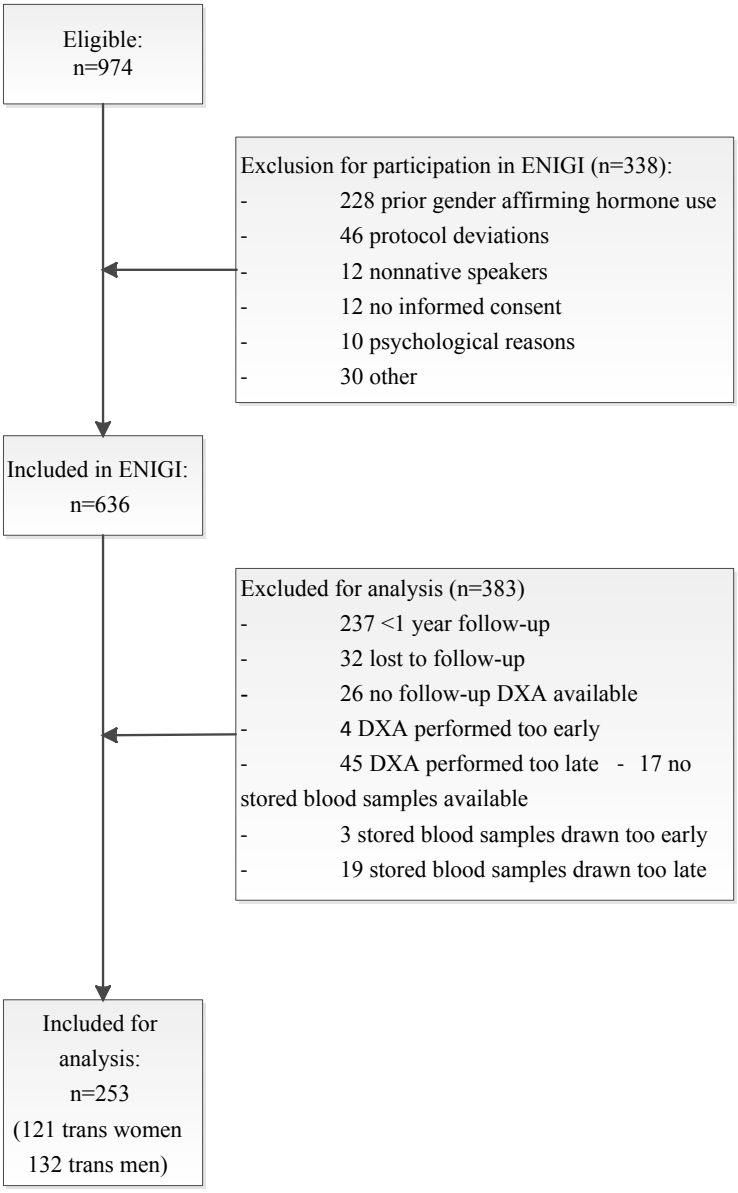

Figure 1. Flowchart of in- and exclusion of participants. ENIGI = European Network for Investigation of Gender Incongruence.

Table 1. Participant characteristics at baseline and after 1 year of HT

	Trans women (n=121)		Trans men (n=132)	
	Baseline	**1 year of HT**	**Baseline**	**1 year of HT**
General				
Age, yr (median, IQR)	30 (24 – 41)		24 (21 – 33)	
Ethnicity (% Caucasian)	96.7		91.7	
BMI, kg/m^2 (median, IQR)	22.9 (20.8 – 26.1)	24.1 (21.9 – 26.3)	24.5 (21.4 – 29.0)	25.4 (22.5 – 29.2)
Tobacco use (% yes)	24.0	14.0	29.2	20.0
- Cigarettes/day (median, IQR)	10 (5 – 10)	6 (4 – 20)	8 (4 – 15)	7 (3 – 15)
Alcohol use (% yes)	46.3	45.6	51.2	47.5
- Units/week (median, IQR)	2 (1 – 5)	2 (2 – 4)	2 (1 – 4)	3 (2 – 5)
Biochemical results (median, IQR)				
Estradiol, pmol/L	105 (84 – 133)	204 (137 – 328)	187 (67 – 525)	181 (132 – 261)
Testosterone, nmol/L	19.0 (14.0 – 23.0)	0.7 (0.5 – 1.0)	1.3 (1.0 – 1.7)	29 (20 – 39)
LH, U/L	3.2 (2.3 – 4.3)	0.1 (0.1 – 0.1)	5.0 (2.7 – 6.9)	1.5 (0.2 – 3.6)
25OHD, nmol/L	39 (25 – 57)	60 (40 – 76)	54 (30 – 77)	57 (41 – 80)
Creatinine, µmol/L (mean ± SD)	77 ± 10	73 ± 10	66 ± 10	79 ± 12
AST, U/L	24 (20 – 28)	20 (17 – 23)	21 (19 – 25)	24 (20 – 28)
ALT, U/L	22 (16 – 30)	21 (15 – 27)	17 (13 – 24)	22 (17 – 29)
γGT, U/L	20 (15 – 28)	19 (15 – 26)	15 (12 – 23)	17 (12 – 26)

HT = gender affirming hormonal treatment, IQR = interquartile range, LH = luteinizing hormone, AST = aspartate transaminase, ALT = alanine transaminase, γGT = gamma-glutamyltransferase

Trans women

ALP, CTx, and sclerostin decreased with 19% (95%CI -21 to -16), 11% (95%CI -18 to -4), and 8% (95%CI -13 to -4), respectively, in the unadjusted model after 1 year of HT (Table 2). Adjusting the percentage changes in all BTMs for changes in BMI, smoking habits, alcohol use, 25OHD, creatinine, AST, ALT, and γGT concentrations did not affect the results (Table 2). No difference between the different age groups in change in BTMs were found (Figure 2). Sclerostin decreased in all but the lowest estradiol quartile (Figure 3).

Trans men

P1NP, ALP, and sclerostin increased with 33% (95%CI 24 to 42), 16% (95%CI 12 to 20), and 15% (95%CI 10 to 20), respectively, after 1 year of HT (Table 2). Adjusting these percentage changes in BTMs for changes in BMI, smoking, alcohol use, creatinine, 25OHD, AST, ALT, and -GT did not affect the results (Table 2). More detailed analyses based on the earlier specified age groups revealed an opposite effect on bone turnover in the trans men aged ≥50 years after 1 year of HT compared to the younger trans men (Figure 2). In trans men aged ≥50 years, a decrease in P1NP of -19% (95%CI -35 to -4), CTx of -32% (95%CI -50 to -13), and sclerostin of -10% (95%CI -19 to -0) were found. Estradiol concentrations increased more in the trans men aged ≥50 years (median increase of 135 pmol/L, IQR 100 to 164) compared to trans men <50 years (median increase of 30 pmol/L, IQR -336 to 124). Different absolute

Table 2. Baseline and 1 year concentrations of bone turnover markers and BMD with corresponding percentage change (mean and 95%CI), for trans women and trans men separately.

	Baseline	1 yr HT	Percentage change %	Percentage change % adjusted [a]
Trans women				
Bone turnover markers				
P1NP, µg/L (median, IQR)	50 (42 – 65)	48 (38 – 62)	-3 (-9 ; 3)	-8 (-17 ; 1)
18-30 years	61 (49 – 74)	52 (47 – 75)	-2 (-10 ; 6)	n.a.
30-50 years	48 (38 – 52)	46 (35 – 54)	+2 (-10 ; 14)	n.a.
≥50 years	40 (33 – 43)	29 (22 – 39)	-15 (-29 ; -1)	n.a.
ALP, U/L (mean ± SD)	70 ± 17	57 ± 17	-19 (-21 ; -16)	-21 (-25 ; 18)
18-30 years	72 ± 19	60 ± 18	-17 (-21 ; -13)	n.a.
30-50 years	69 ± 16	53 ± 13	-23 (-27 ; -19)	n.a.
≥50 years	67 ± 13	58 ± 19	-14 (-24 ; -4)	n.a.
CTx, ng/L (median, IQR)	428 (306 – 538)	329 (265 – 442)	-11 (-18 ; -4)	-11 (-23 ; 1)
18-30 years	507 (387 – 658)	351 (309 – 476)	-17 (-26 ; -9)	n.a.
30-50 years	371 (275 – 500)	313 (265 – 452)	-1 (-17 ; 14)	n.a.
≥50 years	287 (198 – 369)	224 (165 – 279)	-12 (-32 ; 7)	n.a.
Sclerostin, pmol/L (median, IQR)	10.4 (8.6 – 14.9)	8.8 (7.3 – 13.5)	-8 (-13 ; -4)	-9 (-16 ; -2)
18-30 years	8.8 (7.7 – 11.0)	7.7 (6.6 – 9.4)	-8 (-15 ; -0)	n.a.
30-50 years	11.4 (9.4 – 15.0)	11.0 (8.1 – 13.4)	-9 (-15 ; -2)	n.a.
≥50 years	17.7 (16.0 – 21.9)	17.9 (14.1 – 18.5)	-10 (-22 ; 2)	n.a.
DXA				
BMD TH g/cm^2 (mean ± SD)	0.938 ± 0.137	0.947 ± 0.137	+1.0 (0.5 ; 1.5)	+0.8 (0.1 ; 1.6)
BMD FN g/cm^2 (mean ± SD)	0.797 ± 0.127	0.812 ± 0.129	+1.9 (1.3 ; 2.5)	+1.6 (0.7 ; 2.5)
BMD LS g/cm^2 (mean ± SD)	0.968 ± 0.139	1.004 ± 0.138	+3.8 (3.1 ; 4.6)	+3.2 (2.0 ; 4.4)
Trans men				
Bone turnover markers				
P1NP, µg/L (median, IQR)	56 (43 – 71)	71 (49 – 100)	+33 (24 ; 42)	+29 (11 ; 48)
18-30 years	60 (50 – 77)	85 (67 – 111)	+42 (30 ; 54)	n.a.
30-50 years	40 (36 – 52)	53 (37 – 60)	+21 (10 ; 33)	n.a.
≥50 years	46 (41 – 66)	41 (29 – 55)	-19 (-35 ; -4)	n.a.
ALP, U/L (mean ± SD)	67 ± 19	76 ± 22	+16 (12 ; 20)	+15 (7 ; 23)
18-30 years	68 ± 20	80 ± 23	+19 (14 ; 24)	n.a.
30-50 years	62 ± 18	68 ± 17	+14 (5 ; 24)	n.a.
≥50 years	72 ± 21	65 ± 23	-12 (-24 ; 1)	n.a.
CTx, ng/L (median, IQR)	423 (323 – 533)	432 (313 – 529)	+3 (-4 ; 10)	-5 (-19 ; 8)
18-30 years	448 (384 – 590)	442 (364 – 586)	+3 (-4 ; 11)	n.a.
30-50 years	297 (222 – 386)	313 (215 – 387)	+12 (-6 ; 30)	n.a.
≥50 years	427 (305 – 547)	222 (193 – 381)	-32 (-50 ; -13)	n.a.
Sclerostin, pmol/L (median, IQR)	8.7 (6.8 – 13.1)	10.3 (7.9 – 13.2)	+15 (10 ; 20)	+10 (-0 ; 20)
18-30 years	7.6 (6.5 – 9.5)	8.8 (7.4 – 11.3)	+20 (13 ; 26)	n.a.
30-50 years	13.9 (8.8 – 16.8)	14.3 (10.8 – 18.1)	+10 (1 ; 19)	n.a.
≥50 years	15.9 (14.8 – 17.8)	15.3 (13.2 – 16.3)	-10 (-19 ; -0)	n.a.

Table 2. (continued)

	Baseline	1 yr HT	Percentage change %	Percentage change % adjusted [a]
DXA				
BMD TH g/cm^2 (mean ± SD)	0.948 ± 0.113	0.956 ± 0.114	+0.9 (0.4 ; 1.4)	+0.0 (-0.9 ; 0.9)
BMD FN g/cm^2 (mean ± SD)	0.833 ± 0.116	0.825 ± 0.116	-0.9 (-1.6 ; -0.1)	-2.5 (-3.7 ; -1.2)
BMD LS g/cm^2 (mean ± SD)	1.026 ± 0.125	1.036 ± 0.129	+1.0 (0.4 ; 1.7)	+2.1 (0.9 ; 3.4)

HT = gender-affirming hormonal treatment, IQR = interquartile range, ALP = alkaline phosphatase, n.a. = not applicable.

[a] adjusted for changes in BMI, alcohol and tobacco use, 25OHD, creatinine, AST, ALT, and -GT. Data only shown for the total adjusted group, as separate adjusted age groups resulted in too small groups for multivariable analyses.

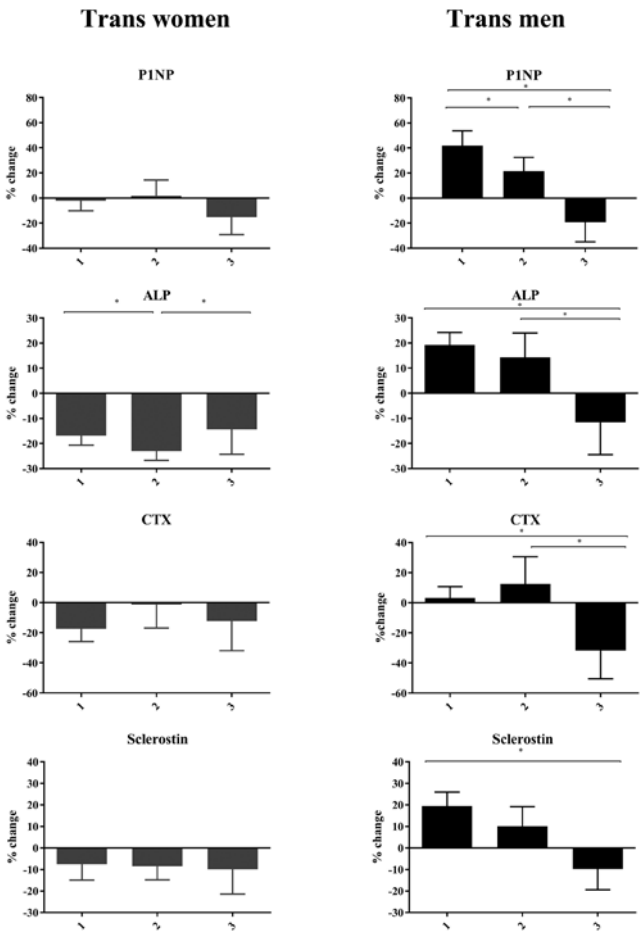

Figure 2. Percentage change in bone turnover markers in trans women and trans men after 1 year of HT, stratified for age groups. Group 1 = 18 to 30 years (trans women mean age 24 (2.9 SD), n = 61, trans men mean age 23 (3.0 SD), n = 91). Group 2 = 30 to 50 years (trans women mean age 39 (5.2 SD), n = 42, trans men mean age 39 (5.9 SD), n = 32). Group 3 = group ≥50 years (trans women mean age 56 (5.8 SD), n = 18, trans men mean age 54 (4.1 SD), n = 9). ALP = alkaline phosphatase. * p ≤ 0.05.

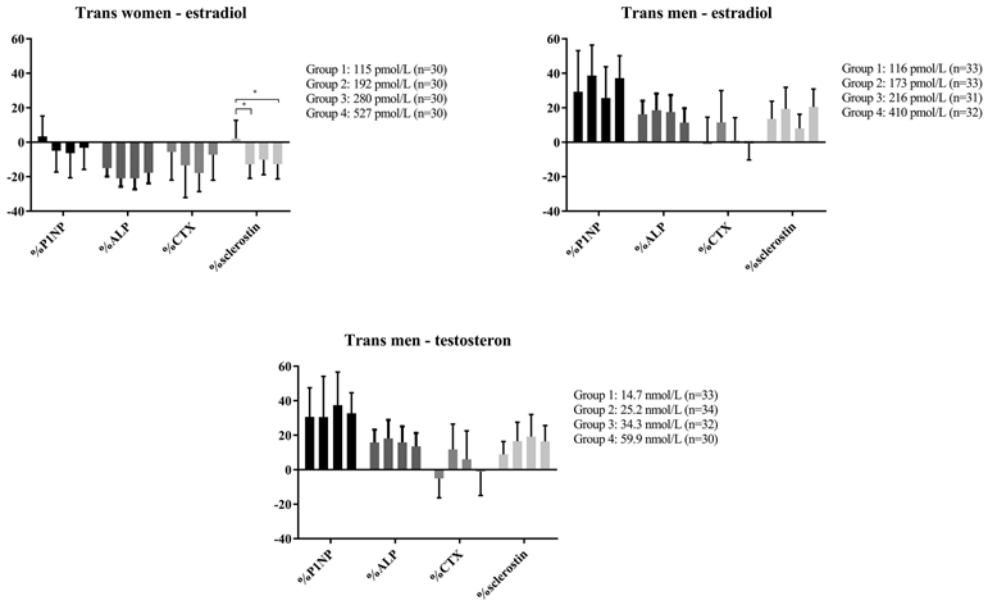

Figure 3. Percentage change in bone turnover markers by quartiles of average estradiol and testosterone concentrations measured at 3 and 12 months after baseline. Testosterone concentrations in trans women were <2 nmol/L, and therefore this group was not further divided into subgroups. ALP = alkaline phosphatase. * $p \leq 0.05$.

concentrations of testosterone and estradiol concentrations during HT did not result in different effects in the course of BTMs during 1 year of HT (Figure 3).

Correlations between BTMs and BMD

Correlations between percentage change in BTM and percentage change in BMD for trans women and trans men are displayed in Table 3. The changes in BMD after 1 year of HT in this transgender population was described extensively in earlier research (5). In trans women, an increase in sclerostin was associated with a decrease in TH BMD. No correlations between change in BTMs and FN BMD were seen. Furthermore, P1NP, ALP, and CTx showed a modest negative correlation with LS BMD after 1 year of HT. In trans men, only P1NP showed a modest negative correlation with TH and FN BMD. Lastly, CTx showed a modest negative correlation with LS BMD in trans men (Table 3). Lastly, subgroup analyses were performed based on the baseline LS BMD data, which we divided into tertiles. This resulted in a mean (± SD) BMD of group 1 (0.817 ± 0.065), group 2 (0.972 ± 0.036), and group 3 (1.120 ± 0.082) in trans women. In trans men, this resulted in a mean BMD (± SD) of group 1 (0.893 ± 0.050), group 2 (1.017 ± 0.029), and group 3 (1.167 ± 0.075). Based on these tertiles, we evaluated changes of bone turnover markers per tertile. This resulted in a decrease of P1NP, CTx, ALP, and sclerostin in trans women, which was similar for all tertiles. In trans men, an increase of all markers except CTx was found, which was similar for the tertiles. In CTx, an increase was found in all but the highest BMD group (group 3).

Table 3. Correlation between percentage change in bone turnover markers and percentage change in BMD (mean and 95%CI), separately for trans women and trans men..

Trans women	TH BMD %	FN BMD %	LS BMD %
P1NP %	-0.10 (-0.27 ; 0.09)	-0.15 (-0.32 ; 0.03)	**-0.28 (-0.44 ; -0.11)**
ALP %	-0.02 (-0.19 ; 0.16)	0.00 (-0.18 ; 0.18)	**-0.22 (-0.38 ; -0.04)**
CTx %	-0.08 (-0.25 ; 0.10)	-0.17 (-0.34 ; 0.01)	**-0.27 (-0.43 ; -0.10)**
Sclerostin %	**-0.21 (-0.38 ; -0.03)**	-0.02 (-0.20 ; 0.17)	0.03 (-0.15 ; 0.21)
Trans men	**TH BMD %**	**FN BMD %**	**LS BMD %**
P1NP %	**-0.21 (-0.37 ; -0.04)**	**-0.20 (-0.36 ; -0.02)**	-0.15 (-0.32 ; 0.02)
ALP %	-0.12 (-0.29 ; 0.06)	-0.05 (-0.23 ; 0.12)	-0.12 (-0.29 ; 0.05)
CTx %	-0.09 (-0.26 ; 0.09)	-0.11 (-0.28 ; 0.06)	**-0.21 (-0.38 ; -0.04)**
Sclerostin %	0.08 (-0.10 ; 0.25)	-0.04 (-0.21 ; 0.14)	-0.08 (-0.25 ; 0.10)

ALP = alkaline phosphatase
Bold text indicates p ≤ 0.05

Discussion

This study evaluates changes in a variety of BTMs, sclerostin, and its correlation with changes in BMD in transgender people during the first year of HT. In trans women, a decrease in bone turnover was seen during the first year of HT, irrespective of age. In trans men, bone turnover increased in the younger groups, and decreased in the oldest trans men. No differences were seen between the different estrogen concentrations and percentage change in BTMs. Lastly, BTMs showed some modest negative correlations with predominantly changes in LS BMD of trans women.

Effects on bone turnover after one year of HT

Trans women

This is the first study to evaluate sclerostin concentrations in transgender people. It is known that sclerostin concentrations are higher in men than women and sclerostin increases gradually with age in both sexes [41]. We found that sclerostin decreased in trans women after 1 year of HT. Previous research suggested that estrogen results in a decrease in sclerostin, which is thought to result in an increase in BMD, as sclerostin is an important inhibitor of the anabolic Wnt/−catenin signaling pathway in osteoblasts [28,29]. An earlier study in premenopausal estrogen-sufficient women did not show changes in serum concentrations of sclerostin during their menstrual cycle and also did not show a relationship with estradiol concentrations [42]. Withdrawal of estrogens however, resulted in an increase in sclerostin in both post-menopausal women and elderly men, suggesting an inverse association between sclerostin and estrogen concentrations [43]. A longitudinal study in Japanese women also showed a decrease in estrogen and increase in sclerostin concentrations during menopause, which resulted in increased bone resorption [7]. The current study indeed showed a decrease in sclerostin in trans women after 1 year of HT. This finding provides additional evidence that estrogen treatment results in a decrease in sclerostin concentrations which has beneficial

effects on bone turnover. This finding aligns well with another study showing that treatment of postmenopausal women with the SERM raloxifene suppresses sclerostin [44].

The finding that CTx decreased during HT is also in line with the hypothesis that the increase in estrogen concentrations reduces osteoclast activity and thereby inhibits bone resorption. Although one study found no change in CTx due to HT in trans women [22], two other studies also found a decrease in CTx concentrations within 2 years of HT and lower CTx concentrations compared to control men after 8 years of HT [23,45]. Furthermore, ALP decreased during HT. A decrease in ALP was earlier found within the first year of HT [15] and during longer follow up [12]. Earlier studies also showed a decrease in P1NP within 2 years of HT [45], and lower P1NP concentrations after 8 years of HT compared with control men [23], while one study showed no changes in P1NP after 3 years of HT [22]. Lastly, the lowest estradiol quartile showed opposite or even no changes in BTMs compared to the other three estradiol quartiles in trans women, which implies that the estrogen concentrations in the lowest quartile might be too low to result in a decrease in bone turnover. Overall, the decrease in BTMs in trans women further support the bone preserving role of estrogens.

Trans men

Sclerostin increased in the younger trans men after 1 year of HT. The effect of androgens on sclerostin concentrations are not fully elucidated yet. An earlier study found a possible direct androgen receptor-mediated effect on the production of sclerostin and negative correlation between sclerostin concentration and testosterone concentrations in birth-assigned men [46]. However, the current study did not show a decrease in sclerostin in trans men who had higher testosterone concentrations after 1 year of HT. On the other hand, another study showed that predominantly estrogen and not testosterone mediated the decrease of sclerostin [43]. However, as both testosterone and estradiol concentrations changed in trans men, we were not able to determine the isolated effect of testosterone. Also, this result can be explained by the use of different sclerostin assays in previous literature with sometimes high variability between various sclerostin assays [47].

With regard to bone formation, an increase in P1NP and ALP was seen after 1 year of HT. An earlier prospective study also showed an increase in P1NP, in respect of no changes in control women [24]. Furthermore, P1NP concentrations in trans men aged 37±8 years were approximately 25% higher compared to control women after 10 years of HT [24], which is comparable to the 21% change reported in our study in this age group. Regarding ALP, earlier studies showed an increase of approximately 13% in ALP within the first year of HT in trans men aged 16-40 years [15], which is also in line with the 13% change reported in our study. A previous study in postmenopausal women showed that ALP concentrations were higher compared to premenopausal women, and that ALP was negatively correlated with the estradiol concentration of the postmenopausal group [48]. The bone specific alkaline phosphatase fraction (BALP) instead of total ALP is a more sensitive parameter to evaluate bone formation, as increased serum ALP can also result from liver or gallbladder disease [38]. However, the trans men did not show signs of liver disease as all liver parameters besides ALP did not change during 1 year of HT, so it is not expected that this affected current results. As muscle

mass increased in trans men, which is resembled by an increase in creatinine after 1 year of HT, mechanical loading on bones increased, which possibly explains the increase in bone formation markers [31]. Concerning bone resorption, current study showed no increase in CTx in trans men after 1 year of HT. Earlier studies in trans men showed an increase in CTx after 1 year HT, compared to no changes in control women [20]. Also, trans men had higher CTx concentrations compared to control women after 10 years of HT [24]. CTx was measured in fasting state, just as in the studies mentioned before. As CTx is cleared by the kidney [38] higher concentrations of CTx can be found in case of impaired kidney function, yet our study population had no impaired kidney function. Alternatively, fasting state was based on self-report of the participants during follow-up. Therefore it is possible that some participants did not apply to the instructions to draw blood in fasting state. This might have masked the increase of CTx as CTx decreases due to food ingestion [38]. Lastly, when comparing age groups, the oldest trans men group showed a decrease in all BTMs and sclerostin in contrast to the younger trans men. The older group of trans men benefited most of HT as they were assumed to be estrogen deficient due their postmenopausal state at baseline (mean age 54 years, SD 4.1). In most studies in trans men, estradiol concentrations either remain stable or decrease slightly. However, two studies investigating the effect of testosterone in combination with an aromatase inhibitor found that estradiol concentrations remained stable in trans men using testosterone only, but decreased to great extent in trans men using both testosterone and aromatase inhibitor [49,50]. This indicates that the estradiol concentrations mainly result from aromatization of testosterone into estradiol. This is also supported by our finding that estradiol concentrations increased in trans men who were postmenopausal and therefore estrogen deficient before the start of HT. The increase in estrogen concentration after aromatization of testosterone resulted in decreased bone resorption, which further strengthens the beneficial role of estrogen on bone health.

Associations between BTMs and BMD

Modest negative correlations were found between changes in BTMs and changes in BMD during 1 year of HT. This finding is in line with previous research in trans women, where no correlations between CTx and P1NP and volumetric BMD [vBMD] of the radius or tibia where found [22]. In trans men, only an inverse relationship with CTx and P1NP and vBMD at radius and tibia was found [24]. Changes in BTMs were predominantly correlated to the LS BMD of trans women. LS consists mainly of trabecular bone which is more metabolic active compared to the hip, that mainly consists of cortical bone [51,52]. This was not seen in the FN, although this region also contains significant trabecular bone albeit less compared to LS. This finding further emphasize the role of estradiol in maintaining adequate bone homeostasis, which is already studied well in men [53,54]. Also, earlier research about estrogen supplementation therapy in postmenopausal women showed an increase in BMD and a decrease in BMD after discontinuation of estrogen supplementation in a large female cohort [55]. Next to this, a murine model studying ovariectomized mice showed that estrogen therapy had more beneficial effects on bone architecture compared to mice treatment with testosterone alone [56]. Summarized, the increase in BMD after 1 year of HT in both trans women and trans men found in this study emphasizes the beneficial effect of estrogen on bone further.

Strengths and limitations

Data for this study was collected during patient care following a standardized treatment protocol. As a result, this prospective study consisted of a large study population compared to other studies in transgender people, thereby ensuring a study population with a broad variation in age. Other strengths of this study were the use of the same BTM assays, all samples were thawed simultaneously, and all analyses were performed using one lot number. Moreover, this study is the first to evaluate the BTM sclerostin in transgender people. In addition, the same DXA scanner was used both at baseline and during follow-up.

This study also has some limitations. First, no control group was included and therefore changes in time as cause for changes in BTMs or BMD could not be evaluated. However, as the study population consisted of different age groups, almost all participants already had reached their peak bone mass and this would have resulted in decreasing BMD through time and increased bone turnover especially in postmenopausal women. From earlier literature it is known that bone turnover increases with age, after the initial high levels that are reached during puberty [57-59]. As part of standard patient care, participants were advised on healthy lifestyle and maintaining adequate calcium and 25OHD intake and physical activity. This resulted in changes in 25OHD concentrations during 1 year HT, but adjustments for these changes did not affect our results. No full data on earlier dietary calcium intake, weight-bearing exercise, steroid use, fractures, or family history were available. Furthermore, a three month measurement of bone turnover markers was not available. Lastly, due to the observational character of this study, this study was not designed to evaluate possible causal relationships. Nevertheless, this study contributes further to the current hypothesis that sclerostin indeed is a mediating factor in the anabolic effect of estradiol on bone turnover and BMD. Lastly, follow-up data regarding fractures were lacking.

To conclude, this study provides additional knowledge regarding the effect of HT on bone metabolism and BMD in transgender people and emphasizes the importance and beneficial effect of estrogen by decreasing bone turnover and increasing BMD. Summarized, this study shows that 1 year of HT does not result in deleterious effects on bone health in transgender people. Despite these results, effects after multiple years of HT, particularly for younger trans men, are of great interest to study in the future. Given the still increasing incidence and need for treatment of transgender people, additional studies should therefore be performed to evaluate the longer term relationships between change in bone turnover, BMD, and fracture risk during HT in transgender people.

Disclosures

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